Twenty Years of Progress in Oncolytic Virus Clinical Trials

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Abstract: In the past few decades, immunotherapy has been considered one of the most promising cancer treatments. Oncolytic virus therapy is one of the major breakthroughs in immunotherapy. Oncolytic viruses are defined as genetically engineered or naturally occurring viruses that selectively replicate and kill cancer cells without harming normal tissues. In this review we present systematic review of 108 clinical trials on oncolytic viruses during the past 20 years. We focus on reviewing trials for their targeted cancer type, methods of virus administration, adverse effect and safety examination, antitumor immune response, antiviral immune response and antitumor activity. Oncolytic virus backbones and delivery methods of the five most common cancers were also evaluated.

1. Introduction

Combating cancer via immunotherapy is a vigorous research focus in cancer treatment which had success fully introduced various immune checkpoint inhibitors and CAR-T cell therapy to the market. Recently, oncolytic virus therapy has been recognized as another major promising option to treat cancer in which the virus replicates specifically in cancer cells, killing them without harming normal cells. The released virus will spread to other cancer cells nearby and demolish the tumor [1]. Oncolytic virus not only destroys cancer cells by direct cytocidal effects, the demolished tumor cells also generate space that allow infiltration of immune cells such as T-cell, NK cell, and APC cell which naturally target cancerous cells and lead to apoptosis [2]. This means that oncolytic virus therapy can also increase patient's own immune response towards tumor [3]. Hence, oncolytic virus as an immunotherapeutic tool has gained enormous attention since it was first explored.

Many oncolytic viruses have undergone clinical trials for cancer treatment. Here we review collective data from clinical trials of oncolytic virus conducted during recent 20 years and analyzed the efficacy, study design and safety evaluation of oncolytic virus clinical research with the aim to give overview of current status of clinical trial of oncolytic virus on cancer treatment and may provide insight on better clinical research design. Furthermore, we predicted the oncolytic virus future development according to findings in clinical trials.

2. Method

Our literature review was conducted by searching PubMed database using keywords "Oncolytic viruses" and "Oncolytic viral" and collected literatures published between 2000, November – 2021, November. 138 articles were firstly identified as relevant manuscripts. We cleaned up this preliminary article pool by rejecting review articles, reports which contain clinical protocol only, and papers which conducted experiments on animal models. 108 manuscripts were kept for further review in this study.

12 critical factors from clinical trials have been reviewed for these 108 articles. Data-wrapper online tool was applied for data visualization. As a retrospective review of published clinical trials,

our statistical analyses were descriptive in nature. Groups were classified for easier presentation in some cases.

3. Oncolytic viruses in clinical research

3.1 Clinical trial phases

Among the 108 selected studies, most of the studies are mainly from clinical trials at early stages. There are 63 phases I clinical trials, which mainly focus on the safety or delivery efficiency of oncolytic viruses (Fig. 1.A). 15 trials are at phases II which aimed to evaluate the effects of oncolytic viruses, including several comparative studies. There are 10 phase I/II experiments, and only 2 phase III trials, all of which were targeting melanoma. In addition, 18 studies did not mention clinical phases or were non-specific clinical studies.

3.2 Cancer types

In these 108 independent clinical trials we collected, 3405 patients were involved in total. These patients participating in the oncolytic virus clinical trial were classified according to the cancer type. A total of 14 types of cancer have been recorded (Fig. 1.B). Among them, the most targeted cancer is melanoma, accounting for 30% of all patients. The second is digestive/gastrointestinal cancer, which accounts for 19% of all patients. The next three are respiratory/thoracic cancer, genitourinary cancer, and gynecologic cancer, accounting for 8%, 7% and 6% of all patients respectively. The remaining cancer type, which account for less than 5% of all patients, have not been discussed in detail here, including neurologic (4.9%), head and neck (4.5%), breast (2.5%), hematologic/blood (1.8%), musculoskeletal (1.8%), AIDS-related (0.7%), endocrine and neuroendocrine, germ cell and eye cancer. In addition, 13% of patients have non-specific types of cancer, including solid tumors and pediatric tumors.

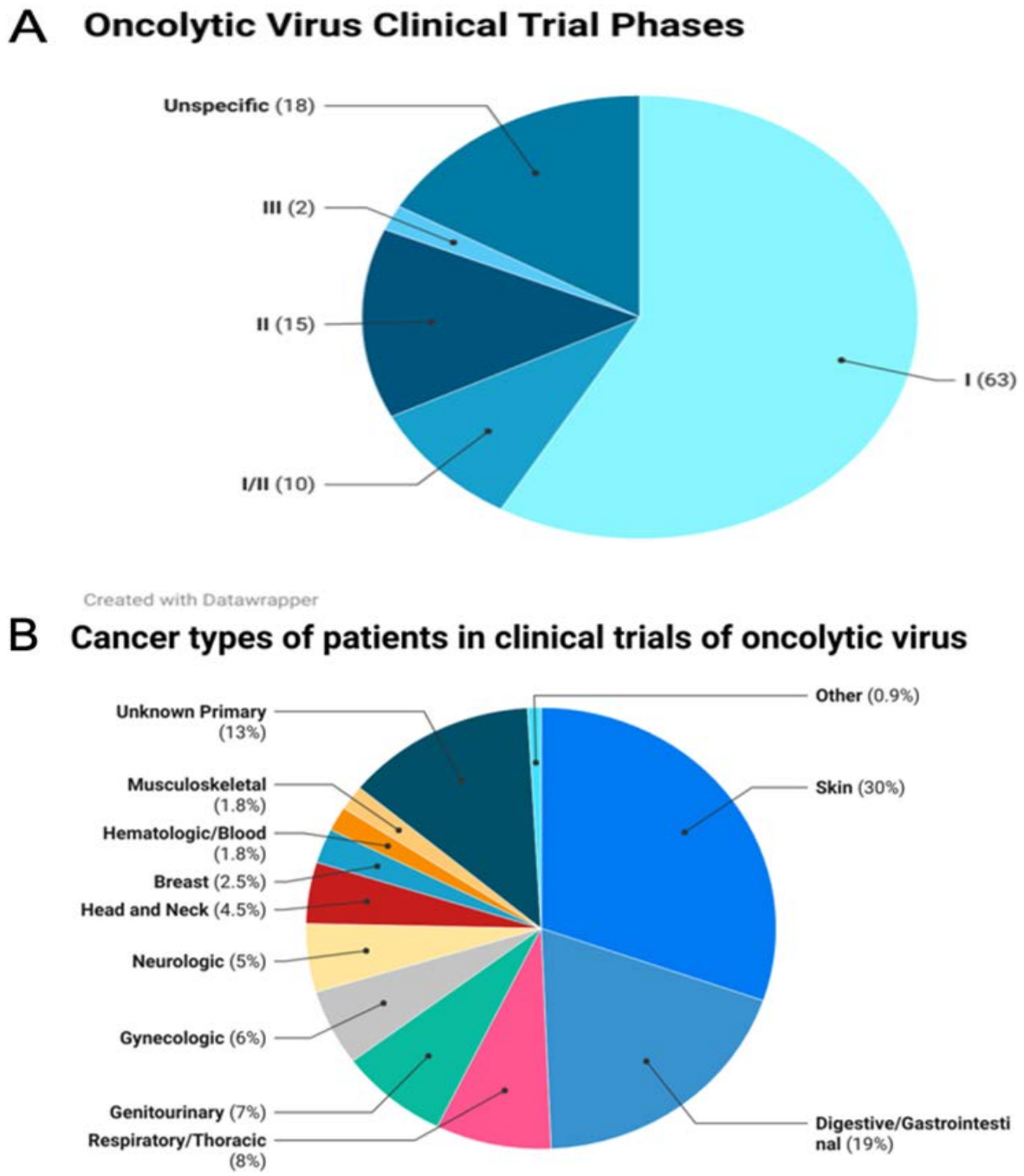

Fig 1. A. Oncolytic virus clinical trial phases. B. Cancer types in oncolytic virus clinical trials.

3.3 Oncolytic virus delivery method

Oncolytic viruses were mainly delivered in 8 ways (Fig. 2.A) in these 108 studies. The most used oncolytic virus delivery strategy is intratumoral/ intralesional, involving 1414 patients in total. Intratumoral/intralesional is currently considered the most effective oncolytic virus delivery strategy as it can reduce the immune system response upon administration. The second most used delivery method is intravenous injection, with a total of 1052 patients participated. Although this delivery efficiency is not as good as the intratumoral/intralesional one, it has the advantage to achieve systemic delivery. The remaining 6 major injection methods are intraperitoneal (60 patients), intravesical (95 patients), intrapleural (13 patients), intradermal (16 patients), hepatic perfusion (83 patients) and combined delivery (81 patients). In addition, the injection method of 23 patients was not specifically reported.

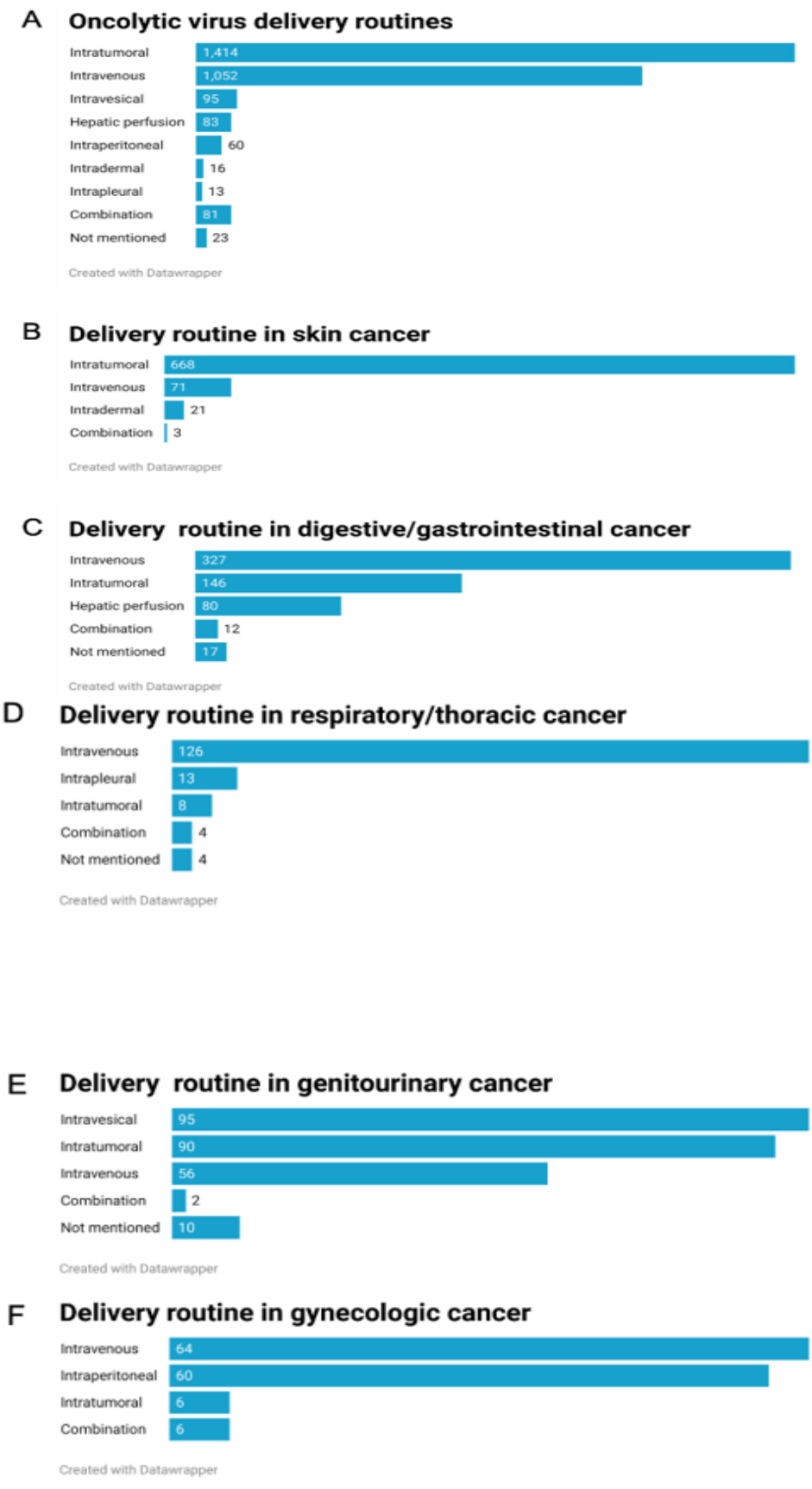

Fig 2. Delivery routines of oncolytic virus clinical trials in different cancers.
A. Viral delivery routines in clinical trials of oncolytic viruses. B. Oncolytic virus delivery routines in skin cancer. C. Oncolytic virus delivery routines in digestive/gastrointestinal cancers. D. Oncolytic virus delivery routines in respiratory/thoracic cancer. E. Oncolytic virus delivery routines in genitourinary cancer. F. Oncolytic virus delivery routines in gy cancer.

3.4 Combination therapy

One of the greatest advantages of oncolytic virus therapy in cancer treatment is that it can be combined with other therapies. Among 108 clinical trial studies, 75 studies were oncolytic virus monotherapy (Fig. 3). The combination of oncolytic virus and chemotherapy were adapted in 21 studies. The combination of oncolytic virus with immunotherapy and radiotherapy were reported in 4 and 2 studies respectively. In addition, several studies have combined three therapeutic approaches, including 5 studies adapting oncolytic viruses, radiotherapy and chemotherapy, and 1 study adapting oncolytic viruses, immunotherapy and chemotherapy.

In the following sections, the clinical trial of oncolytic viruses on top 5 specific cancer types will be described.

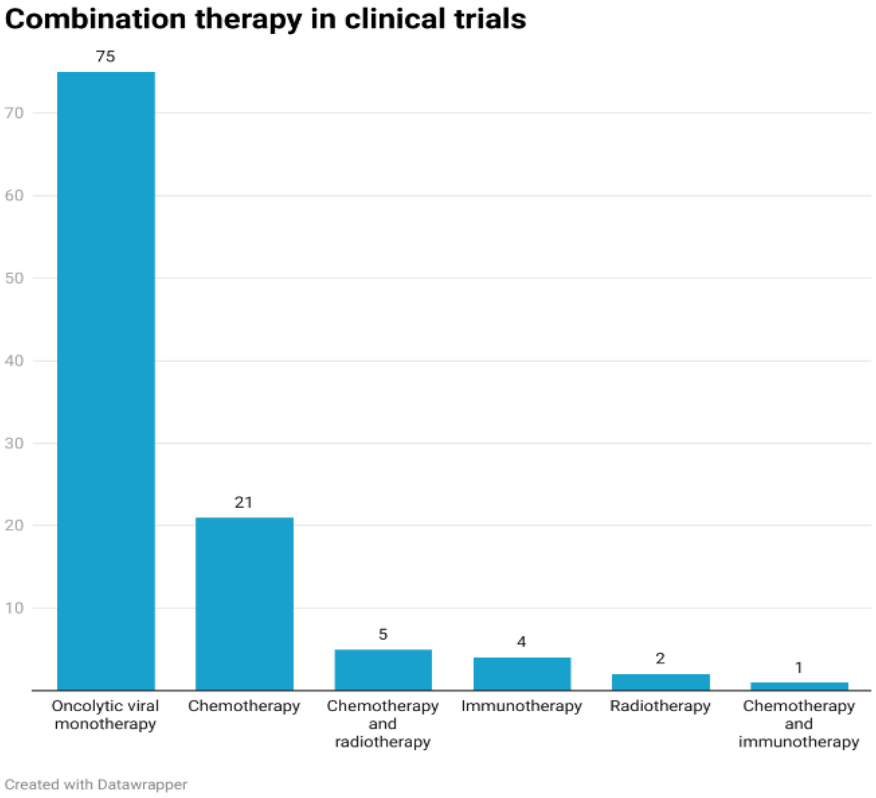

Fig 3. Combination therapy in oncolytic viral clinical trials.

3.5 Skin cancer

Out of 1,030 skin cancer patients participated in clinical trials of the oncolytic virus, 763 patients have received oncolytic virus treatment (Table I). Among these 763 patients, 758 patients were with melanoma, 3 patients were with skin squamous cell carcinoma, 1 patient was with basal cell carcinoma, and 1 patient was without specific type of skin cancer mentioned. Of all 32 clinical trials of oncolytic viruses applied to skin cancer, 16 studies were at phase I; 7 studies were at phase II, 2 were at phase I/II and 2 were at phase III. As the only oncolytic virus that has been approved by the FDA to target melanoma, Talimogene Laherparepvec (T-VEC) is the only therapy that has get into Phase III clinical trials. In addition, five studies did not specify their clinical research phases or were at non-specific clinical studies.

There are four main delivery methods for the clinical trials of oncolytic viruses applied to skin cancer (Fig. 2.B). The most used is intratumoral administration, with a total of 668 patients (87.5%) participated. Another 71 patients received intravenous injections (9.3%), 21 patients (2.8%) received intradermal injections, and three patients received combined delivery.

There are mainly eight backbones of various oncolytic viruses used in melanoma clinical trials (Table I). The most common virus type is the herpes virus, which was received by 620 patients (81.2%), of which 570 patients (74.7%) received HSV-1 as the backbone of the herpes virus. The

second common oncolytic virus type used for skin cancer was reovirus, which was received by 62 patients (8%). The other 6 oncolytic viruses applied are Newcastle disease virus (received by 26 patients (3.4%)), adenovirus (received by 23 patients (3.0%)), poxvirus (received by 15 patients (1.9%)), and fowlpox viruses (received by 10 patients (1.3%)), HVJ (received by 6 patients (0.8%)), and vaccinia (received by 1 patient (0.1%)). Transgene can be engineered into oncolytic virus to enhance the therapeutic effect. Of the 32 skin cancer clinical studies we identified, 13 studies have used GM-CSF as the transgene; two studies have harnessed B7.1 and human telomerase reverse transcriptase gene (hTERT) promoter as transgenes, respectively.

Table 1. Enrollment, phases, and viral backbone of the five most common cancers in clinical trials of oncolytic viruses.

Cancer Type	Patient	Phase	Viral Backbone
Skin cancer	763	I (16); I/II (2); II (7); III (2); Unspecific (5)	Herps virus (81%); Reovirus (8%); Newcastle disease virus (3.4%); Adenovirus (3%); Poxvirus (2%); Fowlpox virus (1.3%); HVJ (0.8%); Vaccinia virus (0.1%)
Digestive/Gastrointestinal cancer	582	I (26); I/II (2); II (5); Unspecific (9)	Reovirus (40%); Adenovirus (16%); Herps virus (14%); Vaccinia virus (14%); Newcastle disease virus (11%); Poxvirus (5%); Fowlpox virus (0.3%)
Respiratory/Thoracic cancer	155	I (11); I/II (1); II (3); Unspecific (3)	Reovirus (54%); Picornavirus (15%); Herps virus (9%); Adenovirus (9%); Newcastle disease virus (8%); Poxvirus (3.9%); Vaccinia virus (1.3%)
Genitourinary cancer	253	I (17); I/II (2); II (2); Unspecific (3)	Adenovirus (69%); Reovirus (9%); Herps virus (6%); Coxsackievirus (6%); Newcastle disease virus (4.3%); HVJ (3.6%); Vaccinia virus (2%)
Gynecologic cancer	136	I (9); II (2); Unspecific (1)	Reovirus (42%); Adenovirus (30%); Measles virus (16%); Vaccinia virus (7%); Newcastle disease virus (4.4%); Poxvirus (1.5%)

3.6 Digestive/Gastrointestinal cancer

653 patients with digestive/gastrointestinal cancer including colon cancer, liver cancer and pancreatic cancer have participated in 42 oncolytic virus clinical trials, 582 of them have received oncolytic virus treatment (Table I). Out of reports from 42 clinical investigations, 26 are from phase I studies; 2 are from phase I/II, and 5 studies are at phase II. In addition, 9 studies are non-specific clinical trials.

The delivery approaches of oncolytic viruses used in digestive/gastrointestinal cancer clinical trials are diverse (Fig. 2.C). The most used method is intravenous injection with 327 patients (56.2%) involved, followed by intratumoral injection with 130 patients (22.3%) involved. The remaining oncolytic virus administration methods are hepatic perfusion (80 patients involved-give out, 13.7%) and combined delivery (12 patients involved, 2.0%). In addition, the delivery method for 17 patients (2.9%) was not specifically reported. It is worth mentioning that some patients received intraperitoneal administration but combined with intravenous (IV) ones because of intraperitoneal disease.

There are mainly eight types of virus backbones applied as the oncolytic virus to target digestive/gastrointestinal cancer (Table I). The most common type is the reovirus, which was received by 230 patients (39.5%). The remaining seven backbones were herps vaccinia virus (received by 80 patients, 13.7%); adenovirus (received by 91 patients, 15.6%); parvovirus (received by 7 patients,

1.2%); poxvirus (received by 32 patients, 5.5%); fowlpox viruses (received by 2 patients, 0.0%) and Newcastle disease virus (received by 64 patients-give out percentage, 11.0%). A total of 9 studies have used GM-CSF as the transgene to enhance the therapeutic effect of oncolytic viruses. In addition, B7.1 and FCU-1 have been used as transgenes by 2 studies respectively, and there are 2 studies applying thymidine kinase (TK) as transgene.

3.7 Respiratory/Thoracic cancer

A total of 270 respiratory/thoracic cancer patients were recruited in 18 oncolytic virus clinical trials, 155 of them have received oncolytic virus treatment (Table I). Among the 18 studies, 11 clinical trials were at phase I; 1 study was at phase I/II; 3 studies were at phase II and 3 studies are non-specific clinical trials. The most common respiratory/thoracic cancer type involved are lung cancer and mesothelioma.

In the respiratory/thoracic cancer clinical trial, the most common oncolytic virus administration routine was intravenous, with a total of 126 patients participated (81.2%) (Fig. 2.D). The remaining delivery methods include intrapleural, intratumoural, and combined delivery. The oncolytic virus administration routine for 4 patients (2.6%) was not specifically mentioned. It is noted that the intrapleural administration routine has only been applied to mesothelioma patients in 1 study.

A total of 6 virus backbones have been used in respiratory/thoracic cancer clinical trials (Table I). Reovirus, the most used respiratory/thoracic cancer oncolytic virus, was applied to 84 patients (54.1%). The remaining backbone used are herpes virus (received by 13 patients (HSV-1), 8.4%); adenovirus (received by 14 patients, 9.0%); picornavirus (received by 24 patients, 15.5%); vaccinia virus (received by 2 patients, 1.2%); Newcastle disease virus (received by 12 patients, 7.7%). 3 of the 17 clinical trials have used GM-CSF as a transgene, and only 1 study have applied hTERT promoter as a transgene.

3.8 Gynecologic cancer

A total of 190 gynecologic cancer patients have participated in oncolytic virus clinical trials, and 136 patients received oncolytic virus treatment (Table I). Of the 15 related clinical trials, 9 were at phase I; 2 were at phase II. 4 studies did not specify their clinical phases.

The oncolytic viruses in these gynecologic cancer clinical trials were mainly delivered in four ways (Fig. 2.E). The most common methods are intravenous and intraperitoneal injection, with 64 (47.1%) and 60 (44.1%) patients involved respectively.

IT injections had been provided for 6 patients (4.4%). The oncolytic virus administration method of 6 patients (4.4%) was not specifically mentioned.

A total of 6 virus backbones have been used in gynecologic cancer clinical trials (Table I). The most common type is the reovirus with a total of 57 patients involved (41.9%). The remaining backbones applied include the vaccinia virus (received by 9 patients, 6.6%); adenovirus (received by 41 patients, 30.1%); poxvirus (received by 2 patients, 1.5%); measles virus (received by 21 patients, 15.4%); Newcastle disease virus (received by 6 patients, 4.4%). The reported transgenes engineered in gynecologic cancer clinical trials include GM-CSF (2 studies) and CEA.

3.9 Genitourinary cancer

In the 108 trials collected, a total of 253 genitourinary cancer patients participated in oncolytic virus therapy clinical trials and all received oncolytic virus treatment (Table I). Among all 24 studies, 17 are phase I studies; 2 are phase I/II studies; and 2 are phase II studies. In addition, 3 studies did not specify their clinical trial phase.

Genitourinary cancer patients receive intravesical delivery as the route of administration most commonly (95 patients involved, 37.5%) (Fig. 2.F). 90 (35.6%) and 56 (22.1%) patients received IT and IV injections respectively. In addition, 2 patients (1.0%) received combined injections, and 10 patients (4.0%) with no route of injection reported. It is noted that all those who received intravesical administration were bladder cancer patients.

7 viral backbones were used in genitourinary cancer clinical trials (Table I). The most common one is the adenovirus with 174 patients (68.8%) involved. The remaining backbone used were HVJ (received by 9 patients, 3.6%); the vaccinia virus (received by 5 patients, 2.0%); the herpes virus (received by 16 patients, 6.3%); the reovirus (received by 23 patients, 9.0%); Newcastle disease virus (received by 11 patients, 4.3%) and the coxsackievirus (received by 15 patients, 5.9%). 4 studies have used GM-CSF as the transgene to enhance the effect of oncolytic viruses. HSV-1 TK, hTERT promoter, and RGD modification have been used in 3 independent trials as transgenes respectively.

3.10 Safety data of oncolytic virus treatment.

Most clinical trials have reported the safety status of the treatment but the way they provided data was slightly different. Figure 4 shows the way they deliver the information about the toxicity of the oncolytic virus clinic trials (Fig. 4.A). The majority of articles demonstrated sides' effects data in detail. 90% of articles observed toxicity of oncolytic virus treatment and 10% of studies did not conduct the safety study of the clinic trial.

5 grades system have been applied by most toxicity studies [4], in which grade 1 indicate mild side effect, grade 2 means moderate side effect, grade 3 corresponds for severe side effect, grade 4 is life-threatening, and grade 5 indicate death. A few studies did not illustrate toxicity in detail. Rather, they reported a few symptoms with the percentage of patients involved or as no side effects over grade 3 were observed. These studies were labeled as 'slightly mentioned' in Figure 4.

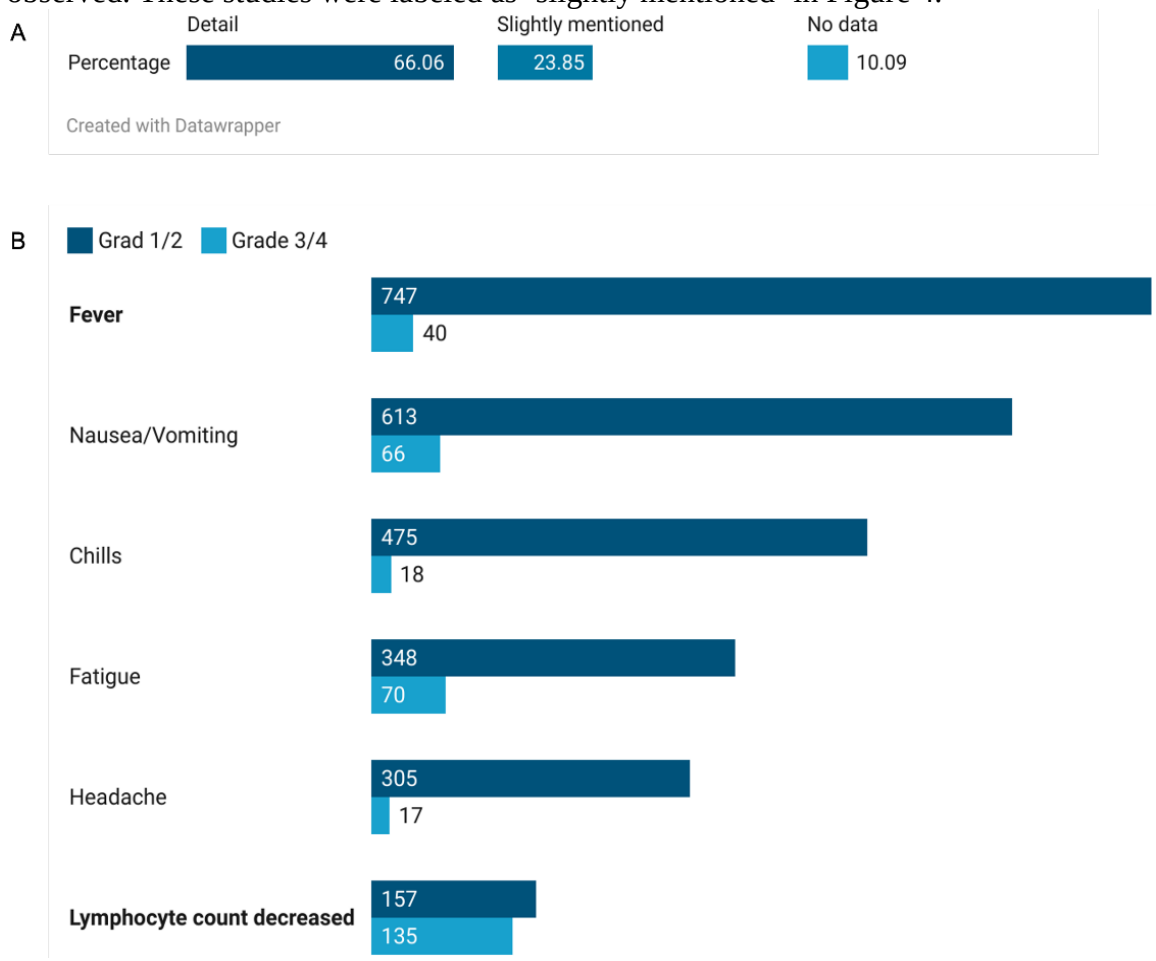

Fig 4. (a) Percentage of clinical trials classified with types of safety data analysis.
(b) 6 common symptoms found in clinical tirals in grade1/2, and grade 3/4.

6 major symptoms were found in safety data of these oncolytic virus clinical trials in treating cancer (Fig 4.B). We classified these symptoms into 2 categories: the first category corresponds to grades 1 and 2 indicating mild & moderate side effects. The second category corresponding to grades 3 and 4, indicating severe side effects. In patients with mild & moderate side effects, fever was the

most common symptom. 747 patients have been reported with fever. The second most common symptom is nausea/vomiting (613 patients reported). Other symptoms including chills (475 cases), fatigue (over 300 cases), headache (over 300 patients), and lymphocyte count decrease (157 cases). However, among patients with severe side effects, lymphocyte count decrease was the most common symptom with 135 patients reported. Other most common symptom for patients with severe side effect (grade 3 and 4) include fatigue (70 patients reported), nausea/vomiting (66 cases), fever (40 patients), and headache and chills were less than 20 cases. From the data, common side effect from oncolytic virus treatment were flu-like symptoms with most cases detected as grade 1 and 2. The lymphocyte count decrease in patients with grade 3 and 4 side effect could reduce the efficiency of oncolytic virus as immune cells are needed to target tumor cells.

3.11 Antiviral immune response in oncolytic virus clinical trials

The immune response from patients against the oncolytic virus could be used as an important biomarker to indicate the activity of oncolytic viruses delivered to patients. 57% of clinical research identified have reported antiviral immunity studies. Among these studies, 3 types of measurement system have been applied to indicate antiviral immune response (Fig 5.A). In the early stage, cytokine, antiviral antibody or immune cell activity have been selected as indicator of early-stage antiviral immune response by different clinical trials. The most used indicator is the virus-specific antibody (51 studies) as it is the most accurate one. The cytokine level or immune cell activity was not used widely as they might increase due to other infections or tumor progress in patients.

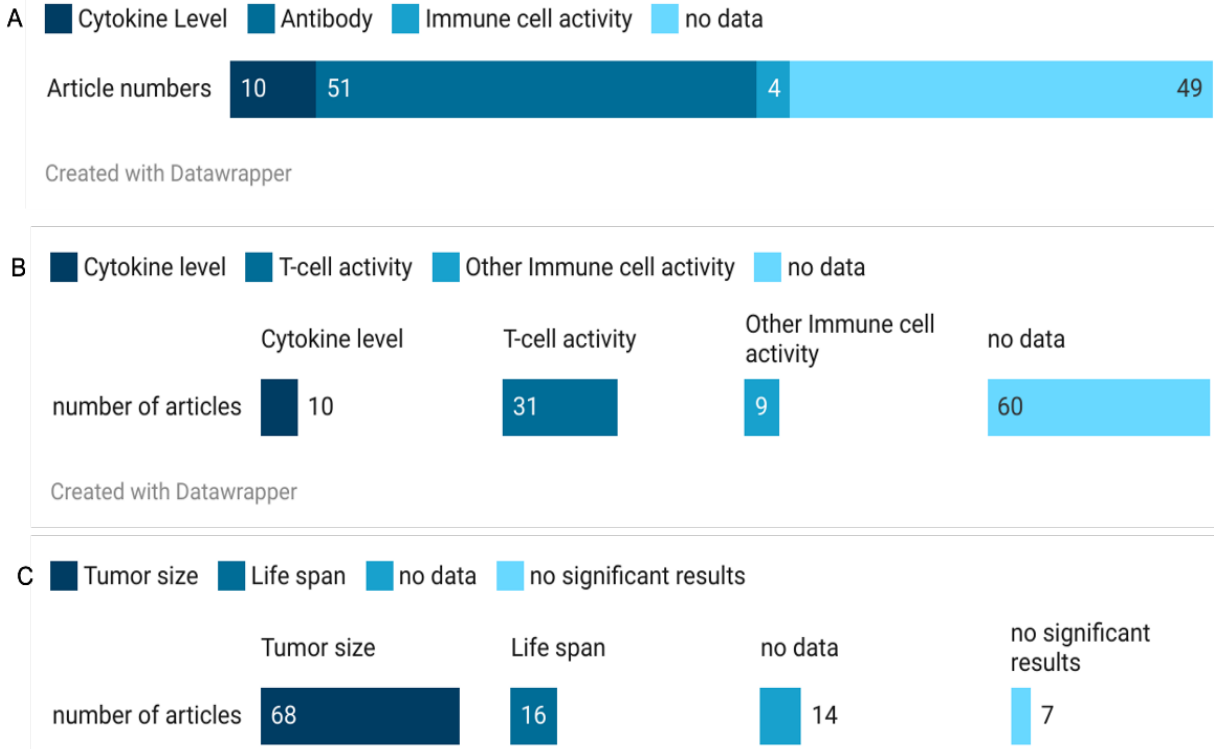

Fig 5. Number of articles classified with antiviral immunity observation.
(b) Number of articles are grouped with antitumor immunity target molecules.
(c) The number of clinical trials is divided by antitumor activity measurement.

3.12 Antitumor immune response in oncolytic virus clinical trials

Oncolytic viruses are expected to activate or enhance antitumor immune response in the patient. 45% of articles studied antitumor immunity in their clinical trials (Fig 5.B). 10 articles measured cytokine level, 31 articles observed T-cell activity after therapy, and 9 research studied other immune cell activity. T-cell activity is the key measurement to indicate the antitumor immune response. The level of cytokine, APC and NK cell activity have been used to refer to T cell activity indirectly. Some studies have found increase of T-cell invasion in tumor after treatment.

3.13 Antitumor activity in oncolytic virus clinical trials.

The antitumor activity measurement is an essential evaluation in oncolytic virus clinical trials to treat cancer. Out of 108 studies, 68 articles have used the tumor size as the read out for antitumor activity; 16 trials used life span to indicate antitumor activity; 7 studies shown no significant result which means no difference between control and experimental group in antitumor activity, and 14 studies did not show antitumor activity data (Fig 5.C). Most studies adopted tumor size measurements as the activity indicator because the lifespan of patients can be affected by other confounders.

Table II presents the data of antitumor reaction measured with tumor size change. The clinical trial having multiple cancer results was not been included to generate the chart. The result was recorded with 4 states which are clinical reaction (CR), partial reaction (PR), stable disease (SD), and progressed disease (PD). Four different cancer types shown CR in clinical trials which are melanoma, bladder cancer, HIV-positive lymphomas, and pediatric cancer. These cancers are highly reactive with oncolytic virus treatment compared to other types of tumors which shown fully cured of the disease in participants. The partial reaction was detected in more cancers including CR observed cancer types. The PR group excludes cancer types in the CR group are malignant glioma, metastatic colorectal cancer, metastatic castration-resistant prostate cancer, pancreatic cancer, epithelial solid tumor, liver tumor, breast cancer, lung cancer, and CTCL. The CR and PR group cancers have high response rate with oncolytic virus treatment. In contrast, some cancers did not show significant reaction with oncolytic viruses. Peritoneal carcinomatosis and ovarian cancer demonstrated SD and PD in trials.

Table 2. Antitumor activity in different types of cancer.

Cancer Type	CR	PR	SD	PD
Malignant glioma	0	34	9	4
Metastatic colorectal cancer	0	22	54	3
Metastatic castration-resistant prostate cancer	0	14	5	0
Melanoma	63	95	56	19
Pancreatic cancer	0	33	82	46
Epithelial solid tumors	0	5	0	0
HIV-positive lymphomas	7	20	0	0
Peritoneal Carcinomatosis	0	0	2	5
Bladder cancer	17	0	0	0
Liver tumors	0	3	14	8
Pediatric cancer	6	6	0	0
Breast cancer	0	2	0	0
Ovarian cancer	0	0	19	2
Lung cancer	0	2	0	0
Lymphomas (CTCL)	0	1	2	2

The number of patients is divided in four different groups which are CR (clinical reaction), PR (partial reaction), SD (stable disease), and PD (progressed disease).

4. Discussion

As a novel immunotherapy developed in recent decades, oncolytic virus immunotherapy has been regarded as one of the most promising cancer treatments. Since Martinus Beijerinck's research led to the discovery of viruses in 1898, viruses have always been one of the hot spots in the medical field. Clinical studies reporting viral infections and tumor regression with some derived clinical trials have led to the theory of viruses as oncolytic agents [5] [6] [7]. With the development of in vitro cell culture technology, cancer biology, and virus-related molecular biology, oncolytic virus immunotherapy has developed from the use of wild pathogenic viruses to alleviate cancer to a variety of native or genetically modified viruses adopted in clinical trials. In this article, 108 human oncolytic virus clinical trials to date have been collected and reviewed. In general, clinical trials of oncolytic viruses

are still in their infancy, and most clinical trials are in phase I to study the safety of oncolytic viruses to target specific cancers. 15 Phase II studies have made preliminary judgments on the therapeutic effects of oncolytic viruses, including comparative clinical trials. For example, Schenk et al. performed randomized double-blind compared the therapeutic effects of oncolytic virus NTX-010 with platinum-based chemotherapy as a most recent phase II clinical trial [8]. In addition, 10 phase I/II studies reported on the safety and preliminary response of oncolytic viruses. However, only 2 phase III clinical trials have been reported, and all of them are related to talimogene laherparepvec (T-VEC) and melanoma. Andtbacka et al. compared effects of T-VEC and GM-CSF in 295 patients with melanoma [9]. After that, they also performed OPTiM clinical trial for 61 patients from original trial which is also the only phase III clinical trial with positive result [10]. Chesney et al. performed T-VEC clinical trials on 41 melanoma patients and recorded information including adverse reactions and viral bioshedding [11]. In addition, 18 studies are non-specific clinical trials. For instance, Parakrama et al. evaluated the characteristics of the colorectal cancer patients' immune response from reovirus [12].

We summarized the types of cancers in 108 clinical trials based on the classification by tumor location provided by the National Cancer Institute. The most common type of cancer the oncolytic virus therapy targeting in clinical trials is skin cancer. Except for a few cases, almost all skin cancers targeted in these trials are melanoma. The first FDA-approved oncolytic virus T-VEC is also targeting melanoma. This may be the reason why melanoma patients account for nearly 30% of all patients. The second most studied cancer type in oncolytic virus therapy is the digestive/gastrointestinal cancer with 42 entries in total.

A variety of oncolytic viruses were selected in clinical trials. Some studies have adopted native virus such as reovirus. A large portion of studies have applied modified viruses to improve virus targeting efficiency and therapeutic response. Transgenes were engineered into the backbone to enhance their therapeutic effects such as in T-VEC. The most adopted transgene is GM-CSF, whose expression can enhance the immune system to kill cancer cells.

As the factor considered to have the greatest impact on oncolytic viruses' efficiency, the delivery method of oncolytic viruses has been diversely applied in clinical trials. In general, the most adopted delivery strategies are intralesional/intratumoral (IT) delivery. The greatest advantage of IT administration is that it enables the direct injection of oncolytic viruses into the tumor, thereby reducing the possibility of impairment of oncolytic viruses' efficacy by the host's immune system [13]. However, oncolytic virus administered by IT delivery is difficult to disperse in tumor microenvironment (TME) and to be delivered systematically due to obstruction in tumor [13]. Some strategies, including junction opening peptides, extracellular matrix enzymes and fusogenic proteins, have been proposed and studied to overcome this problem [14]. However, there are no clinical trials to evaluate whether these strategies can improve the therapeutic efficacy of oncolytic viruses. The second most common administration routine is IV injection which can simultaneously target primary and metastatic cancer cells. Especially the physical condition of some patients cannot support IT injection, this highlights the importance of developing IV administration. Complement factors, antiviral immune cell responses, non-specific viral uptake by macrophages, physical barriers (e.g., blood-brain barrier), dense extracellular matrix, and interstitial fluid pressure all hinder the infiltration of oncolytic viruses [15][16]. There are currently four novel IV delivery routines under development. Tumor microparticles, ultrasound-guided, magnetic drug targeting, and cell vectors are all in the frame, some of which has been adopted in clinical trials [5] [17] [18] [19] [20]. For instance, Ruano et al. adopted mesenchymal cells to deliver oncolytic adenovirus to target solid cancers [21]. In addition to improving delivery efficiency, some methods are explored to directly target the immune system. Roulstone et al. administrated patients an immunosuppressive agent, cyclophosphamide, during IV delivery of reovirus [22]. Although this combination is considered to be safe, it did not effectively attenuate the host's antiviral response. Except for IT and IV, some other delivery routines are favored when targeting certain cancers. For example, bladder cancer and mesothelioma were only taken intravesical and intrapleural administration respectively. In addition, some studies have used

combined injections. For example, Pesonen et al. took a combination of IV and IT injections at different doses based on preclinical data [23].

We collectively reviewed 6 major types of side-effects from oncolytic virus therapy clinical trials. There are also other minor toxicities in clinical trials and unique side-effects observed in certain specific type of cancer. The trial targeting malignant gliomas was one of the examples involving rare side-effects with detected convulsions, partial seizures, mental impairment, photopsia, and somnolence [24]. These brain-related side effects are mainly due to the entry of virus to the brain. Thus, close surveillance is needed to evaluate the toxicity of oncolytic virus therapy targeting critical organs such as the brain. Another side effect is the decrease of lymphocytes after injection which may cause a decreased immune response that leads to decreases of the treatment efficiency [25]. Regarding the antiviral immunity and antitumor immunity studies, not all research recorded these subjects in their oncolytic virus clinical trials. As the principle of oncolytic virus therapy involves viral infection and further triggering of apoptosis of cancer cells, we highly suggest including evaluation of these factors in further clinical trial designs.

5. Further study

As one of the most promising tumor treatment strategies, oncolytic viruses can theoretically be applied to all cancers. In the future, there is a calling for more clinical research on broader range of cancer types other than skin cancers. Comparing to other traditional cancer treatments, oncolytic virus therapy as immunotherapy requires special attention on its activity upon patients' immune system. It is important to find a balance between antiviral immune response and antitumor response. To broaden the scope of the application of oncolytic viruses, it is necessary to investigate patients with overreactive and weak immune systems. Development of delivery approach of oncolytic viruses is another direction to put effort on. To make the delivery of oncolytic viruses more efficient, safer, and more accurate in the future, different carrier-based delivery methods and strategies can be further explored. In addition, oncolytic viruses have excellent potential to combine with other treatments. Therefore, another worthy research direction is modifying oncolytic viruses to enable oncolytic virus therapy to cooperate better with other treatments to achieve the best anti-cancer effect. In conclusion, improving drawbacks of oncolytic virus therapy and combining with other treatment, it could have possibility to be major treatment targeting cancer in future.

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